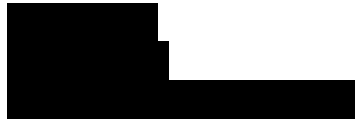




**Refining key lead exposure parameters in Broken Hill – Assessment
of lead relative bioavailability (Phase 1 and 2 report)**

Prepared for: Broken Hill Environmental Lead Program



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Executive Summary

Broken Hill soil samples (BHK1-BHK12) were collected on the 11th of April 2017 along a transect (predominantly along King St) away from the line of lode from Piper St to the laneway behind Knox St. The study aimed to assess the elemental composition of the soil, determine lead (Pb) bioaccessibility using a gastrointestinal extraction method, and for a sub-set of soils (n = 4: phase 1; n = 6: Phase 2), assess Pb relative bioavailability using an in vivo mouse model.

Lead concentration in Broken Hill soil ranged from $215 \pm 0.9 \text{ mg kg}^{-1}$ (BHK8) to $8036 \pm 651 \text{ mg kg}^{-1}$ (BHK1) in the < 2 mm soil particle size fraction. Only two samples (BHK7 and BHK8) were below the National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC) Health-based investigation level (Residential A [HILa]). Lead was enriched in the < 250 μm soil particle size fraction (on average by ~16%) with Pb concentrations ranging from $267 \pm 14 \text{ mg kg}^{-1}$ (BHK8) to $9930 \pm 468 \text{ mg kg}^{-1}$ (BHK1). Enrichment of other elements in the < 250 μm soil particle size fraction was also observed, notably, the concentration of Cd, Zn and Mn exceeded NEPM HILa values in BHK1. Strong relationships were observed between the concentration of Pb and Mn or Zn in the < 250 μm soil particle size fraction suggesting that these relationships may be utilised to ascertain the contribution of outdoor soil to indoor dust Pb concentrations. Assessment of Pb concentration in the < 150 μm soil particle size fraction identified that Pb enrichment occurred for 5 of the 12 soils (BHK1, 5, 6, 7, 12) compared to the < 250 μm soil particle size fraction. When Pb speciation in the < 250 μm soil particle size fraction was assessed using x-ray absorption spectroscopy, Pb was present predominantly as sorbed phases to oxides and clays (45-66%). Lead was also present as plumbojarosite (6-35%), Pb phosphate (5-19%) or bound to organic matter (8-22%).

When Pb bioaccessibility was assessed using the Solubility Bioaccessibility Research Consortium (SBRC) assay, values were significantly higher when determined using gastric phase extraction compared to the intestinal phase. With the exception of BHK4, 8 and 9 (41.5-54.6%), Pb bioaccessibility following gastric phase extraction was > 60% (ranging from 61.7% [BHK12] to 79.5% [BHK10]). However, when gastric phase conditions were transitioned into the intestinal phase, Pb bioaccessibility significantly decreased (by 2.1-21.8 fold) with values ranging from $1.9 \pm 0.2\%$ (BHK8) to $25.3 \pm 1.1\%$ (BHK12). Similar trends in Pb bioaccessibility were observed for the < 150 μm soil particle size fraction although lower intestinal phase Pb bioaccessibility values were derived. Presumably differences in the concentration of elements such as Fe and P in these particle size fractions influenced the outcomes of intestinal phase assessments.

Lead relative bioavailability in Broken Hill soil was assessed using the USEPA's Integrated Systems Toxicology Division mouse model protocol by comparing the accumulation of Pb in target organs (liver, kidney and femur) following a 10 day exposure to Pb-contaminated soil or Pb acetate in AIN93G mouse chow. Mean Pb relative bioavailability ranged from $6.8 \pm 1.2\%$ for BHK3 to $44.8 \pm 5.1\%$ for BHK9, however, for 8 of the 10 soils analysed, mean Pb relative bioavailability was < 20%. Higher Pb relative bioavailability was observed in BHK7 ($33.3 \pm 8.0\%$) and BHK9 ($44.8 \pm 5.1\%$), soils which presented 'low' total Pb concentrations (316 and 452 mg kg^{-1} respectively). For the 10 soils assessed, the upper 95% confidence intervals of the mean for Pb relative bioavailability was 21.5%. Poor relationships were observed when Pb relative bioavailability and Pb bioaccessibility were compared. The gastric phase of the SBRC assay produced values that were up to 10-fold higher (1.2-10.3) compared to the corresponding Pb relative bioavailability value. Although a poor relationship was also observed when the complete data set of SBRC intestinal phase values were compared to Pb relative bioavailability, this in part, was due to the narrow range of Pb relative bioavailability values in Broken Hill soil. For 4 soils (BHK 5, 6, 10, 12), there was no significant difference ($p > 0.05$) between SBRC

intestinal phase values and Pb relative bioavailability while for BHK2, 3, 4 and 11, in vitro assessment over-predicted Pb relative bioavailability by 1.6-3.1 fold. Where elevated Pb relative bioavailability was observed (BHK7, 9), assessment of Pb bioaccessibility using the SBRC intestinal phase, significantly underestimated Pb relative bioavailability.

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Refining key lead exposure parameters in Broken Hill – Assessment of lead relative bioavailability (Phase 1 and 2 report)

Introduction

A key parameter that influences lead (Pb) exposure is Pb relative bioavailability. This parameter controls the ‘effective dose’ that reaches the systemic circulation which then may impart biological effects. Assessment of Pb relative bioavailability (i.e. using an *in vivo* approach) has not been undertaken for Broken Hill soil, however, Pb bioaccessibility (i.e. *in vitro* assessment) has been utilised (e.g. Yang and Cattle, 2015) as a ‘surrogate measure’ of Pb relative bioavailability. Although gastric phase extraction (as utilised by Yang and Cattle) has been recommended by the USEPA for estimating Pb relative bioavailability, there are issues using this methodology when Pb mineralogy is not the controlling parameter for Pb dissolution (a caveat detailed in USEPA, 2012). This will be the case when Pb ore has been weathered or manipulated (i.e. smelting); particularly once Pb emissions have entered the environment, it’s the mineralogy of the environmental matrix that influences dissolution-precipitation events that controls Pb relative bioavailability.

This is exemplified by the scenario at Port Pirie (Solomontown and Port Pirie West) where Pb is predominantly present in soil as sorbed species to mineral and organic components as a consequence of the smelting process (Smith et al., 2011). These species are highly bioaccessible (i.e. almost complete dissolution) when assessed using gastric phase extraction (> 80%) and as a consequence it would be assumed that Pb relative bioavailability is high. When these soils were assessed using an *in vivo* approach (i.e. mouse model), measured Pb relative bioavailability (determined by monitoring blood Pb concentrations) was 30-40% due to the influence of soil iron (Fe) on Pb absorption in the small intestines (Smith et al., 2011). High concentrations of Fe were also solubilised in the stomach, however, when transitioning into the intestines, Fe precipitated (because of the pH change) which in turn facilitated the co-precipitation of Pb. As a consequence, measured Pb relative bioavailability was 2-3 fold lower than values predicted using a gastric phase surrogate assay.

Given that Pb relative bioavailability is a key parameter that defines ‘internal dose’, its assessment is pivotal for quantifying exposure. Although the assessment of Pb relative bioavailability is costly due to the expense associated with *in vivo* studies, this data is critical in order to gain an understanding of exposure, identify factors that influence Pb dissolution and absorption (to inform risk management frameworks) and for establishing the validity of surrogate *in vitro* assays (i.e. bioaccessibility) for its prediction. The final point is significant given that bioaccessibility assays may be a simple, rapid, inexpensive approach for refining exposure variables (on a larger scale) for risk assessment / IEUKB predictions. However, without determining the relationship between *in vivo* Pb relative bioavailability and *in vitro* Pb bioaccessibility, the *in vitro* approach is fraught with danger / uncertainty as its predictive capacity is unknown.

Aims

The aims of this study were to:

1. Identify whether variability in Pb relative bioavailability exists across Broken Hill and its relationship to soil physico-chemical properties (an important parameter for remediation / risk management strategies). If limited variability exists between samples, it may allow for the derivation of a Broken Hill specific default Pb relative bioavailability value for exposure assessment purposes.
2. Determine the relationship between Pb relative bioavailability and Pb bioaccessibility in order to develop a Broken Hill specific in vivo-in vitro linear regression model for predicting Pb relative bioavailability. The 'validated' model would provide confidence in the use of a simple, rapid, inexpensive in vitro approach for refining exposure which is pivotal for IEUKB predictions and to inform a remediation / risk management framework for minimising Pb exposure.
3. Provide a repository of Broken Hill soil with baseline Pb relative bioavailability data which can be utilised for future remediation and risk management research (e.g. soil amendment strategies).

Methods

The overarching research concept of this project was divided into two phases; Phase 1 aimed to characterise collected material (n = 12 soils), assess Pb bioaccessibility (n = 12 soils) and undertake Pb relative bioavailability assessment (n = 4 soils). Following the review of Phase 1 outcomes by the Broken Hill Environmental Lead Program, Phase 2 was initiated which assessed Pb speciation (n = 12 soils) and Pb relative bioavailability in additional soils (n = 6) to support the development of in vivo-in vitro linear regression models.

Soil Sample Collection and Characterisation

In collaboration with the Broken Hill Environmental Lead Program, Pb-contaminated soil (n = 12; ~10 kg / sample) was collected on the 11th of April 2017 for characterisation and bioavailability-bioaccessibility assessment (as detailed below). Surface soil samples (0-5 cm; BHK1-BHK12) were collected in 10 L plastic buckets along a transect (predominantly along King St) away from the line of lode (from Piper St to the laneway behind Knox St). A sub-set of these soils (n = 4; BHK2, 5, 7, 12) were selected for the assessment of Pb relative bioavailability using an in vivo mouse model.

Following collection, soil was air-dried and sieved to recover bulk soil (< 2 mm particle size fraction) and finer fractions (< 250 and < 150 µm particle size fractions). The < 250 µm particle size fraction is currently utilised in Australia to refine exposure (i.e. Tier 2 risk assessment through the provision of bioavailability or bioaccessibility assessment), however, recently the USEPA (2016) revised the soil particle size fraction for bioavailability-bioaccessibility assessment to < 150 µm. All three particle size fractions were assessed for total metal content while the < 250 µm particle size fraction was utilised for Pb relative bioavailability assessment. Bioaccessibility assessment was undertaken on both the < 250 and < 150 µm particle size fractions to determine whether a significant difference in bioaccessibility exists between these fractions. Total metal/metalloid concentration was determined for each particle size fraction by ICP-MS following digestion using USEPA 3051A dissolution procedure (USEPA, 1998). A certified reference material (NIST 2711) was included in the analysis to ensure internal quality assurance/quality control (QA/QC) practices. Soil pH was determined using 1:5 soil:water extracts while total organic carbon was determined by oxidation/combustion (Nelson et al., 1996).

Lead speciation in the < 250 µm soil particle size fraction was determined using x-ray absorption spectroscopy (XAS) at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID, Sector 10, at the Advanced Photon Source (APS) of the Argonne National Laboratory (ANL), U.S. The storage ring operated at 7 GeV in top-up mode. A liquid N₂ cooled double crystal Si(111) monochromator was used to select the incident photon energies and a platinum-coated mirror was used for harmonic rejection. Calibration was performed by assigning the first derivative inflection point of the absorption L_{III}-edge of Pb metal (13035 eV), and each sample scan was collected simultaneously with a Pb metal foil. The samples were ground and pressed into pellets, affixed to a 20-hole sample holder, and mounted for analysis without any further modifications. All sample and standard spectra were calibrated to a Pb foil on the same energy grid, averaged, normalized and the background removed by spline fitting. Principal components analyses were performed in Sixpack on normalized scans, and target factor analyses of each Pb standard were performed to determine the most appropriate standards to be used for linear combination fits analyses.

Assessment of Lead Relative Bioavailability Using an In Vivo Mouse Assay

Lead relative bioavailability in Broken Hill soils was assessed using the USEPA's Integrated Systems Toxicology Division mouse model protocol (IRP-NHEERL-RTP/ISTD). This methodology included a 10 day exposure period (i.e. multiple dosing scheme) while a point estimate approach was utilised to determine Pb relative bioavailability. The point estimate approach (compared to a dose-response approach) was utilised in order to minimise costs and time required to undertake the analysis. Female C57BL/ mice were administered AIN93G purified rodent diet to which Pb (reference dose or Pb-contaminated soil) was added. Twelve mice in four metabolic cages constituted an experimental run. Mice were maintained for 10 days with unlimited access to test diet and drinking water. Cumulative food consumption for each cage was determined as the sum of daily food consumption.

Samples for Bioavailability Assessment

1. ***Lead acetate***: Initially, the response of Pb acetate (15 mg kg⁻¹) in AIN93G mouse chow was assessed. Lead acetate was utilised as it is the lead form from which the Pb toxicity reference value is derived. The Pb acetate response was determined in order to calculate Pb relative bioavailability for Pb-contaminated soils.
2. ***Broken Hill Soil***: Lead relative bioavailability in Broken Hill soil was determined for four (4) locations (BHK2, 5, 7, 12) using a point estimate approach. It is envisaged that Phase 2 activities will build on this Broken Hill Pb relative bioavailability data base through the assessment of an additional four soils. Soil from each location was amended to AIN93G mouse chow (at ~ 1% w/w). Lead relative bioavailability was calculated by comparing the dose normalised accumulation of Pb in target endpoints for Pb in soil versus Pb acetate.
3. ***Soil Reference Material***: USEPA's SoFC-1 was utilised as the reference material for Pb relative bioavailability assessment in soil. SoFC-1 was amended to AIN93G mouse chow (at ~ 1% w/w) while a negative control was also be included to assess 'background' Pb in mouse endpoints.

At the end of the exposure period, mice were euthanized and liver, kidneys and femurs harvested for the determination of Pb accumulation by ICP-MS. Lead relative bioavailability was calculated according the equation 1.

$$\text{Lead Relative Bioavailability (\%)} = \left(\frac{\text{Lead accumulation}_{[\text{soil or dust}]}}{\text{Lead accumulation}_{[\text{Pb-Acetate}]}} \times \frac{D_{[\text{Pb-Acetate}]}}{D_{[\text{soil or dust}]}} \right) \times 100 \quad \text{Equation 1}$$

Where:

Lead accumulation_[soil or dust] = Lead (µg) accumulated in the liver, kidneys and femur following oral administration of Pb-contaminated soil amended mouse chow.

Lead accumulation_[Pb-Acetate] = Lead (µg) accumulated in the liver, kidneys and femur following oral administration of Pb acetate amended mouse chow.

D_[Pb-Acetate] = Dose of Pb administered in Pb acetate amended mouse chow (mg kg⁻¹).

D_[soil or dust] = Dose of Pb administered in Pb-contaminated soil amended mouse chow (mg kg⁻¹).

Assessment of Lead Bioaccessibility Using and In Vitro Approach

The Solubility Bioavailability Research Consortium (SBRC) in vitro assay, incorporating both gastric and intestinal phases (Kelley et al., 2002) was utilised for the assessment of Pb bioaccessibility. Initially, Pb acetate or contaminated soil was combined with gastric phase solution (30.03 g l⁻¹ glycine adjusted to pH 1.5 with concentrated HCl) in polyethylene screw cap flasks. Lead acetate was added to achieve a final concentration of 10-100 mg Pb l⁻¹ while soil was added at a soil:solution ratio of 1:100. After 1 h incubation (37°C, 40 rpm end-over-end shaking), gastric phase samples (10 ml) were collected, filtered through 0.45 µm filters for analysis by ICP-AES. Following gastric phase dissolution, the gastric solution was modified to the intestinal phase by adjusting the pH (to 7.0) with 5 or 50% NaOH and by the addition of bovine bile (1750 mg l⁻¹) and porcine pancreatin (500 mg l⁻¹). After 4 h, intestinal phase samples (10 ml) were collected and filtered through 0.45 µm filters for analysis by ICP-AES or ICP-MS. All gastric and intestinal phase extractions were performed in triplicate for Pb acetate and in duplicate for each soil sample and particle size fraction (< 250 µm and < 150 µm). Absolute Pb bioaccessibility was calculated by dividing the gastric phase extractable Pb (termed SBRC-G) and intestinal phase extractable Pb (termed SBRC-I) by the total Pb acetate or soil Pb concentration. Relative Pb bioaccessibility (termed Rel-SBRC-I) was determined by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at the corresponding pH value (Juhasz et al., 2009; Smith et al., 2011).

Quality Assurance and Quality Control

The accuracy of the aqua-regia digestion method was confirmed by a quantitative average Pb recovery of 1121 ± 34 mg kg⁻¹ (n = 3) from NIST 2711 (1162 ± 31 mg Pb kg⁻¹). During the determination of Pb concentration in soil digests, in vitro extractions and organ samples, duplicate analysis, spiked sample recoveries and check values were included. The average deviation between duplicate samples (n = 20) was 4.3%, the average recovery from spiked samples (n = 20) was 96.9% (93.1-104.1%) whereas check value recoveries (n = 30) ranged from 93.4-109.6% (103.4% average recovery).

Results and Discussion

Soil Collection and Characterisation

Figure 1 details the location of soil samples (BHK1-12) collected along a transect away from the line of lode, predominantly along King St. Soils were sandy in nature (Figure 2), with a low organic carbon content (< 2.8%) and a pH ranging from 6.3 (BHK1) to 7.8 (BHK 7). Particle size distribution, determined using a dry sieving approach, determined that a large proportion of the bulk soil (49.4% [BHK12] to 80.7% [BHK10]) had a particle size of < 250 μm while 9.2% (BHK6) to 24.3% (BHK10) of the bulk soil mass had a particle size < 53 μm , a particle size fraction which may be suspended during high wind events (Figure 3).

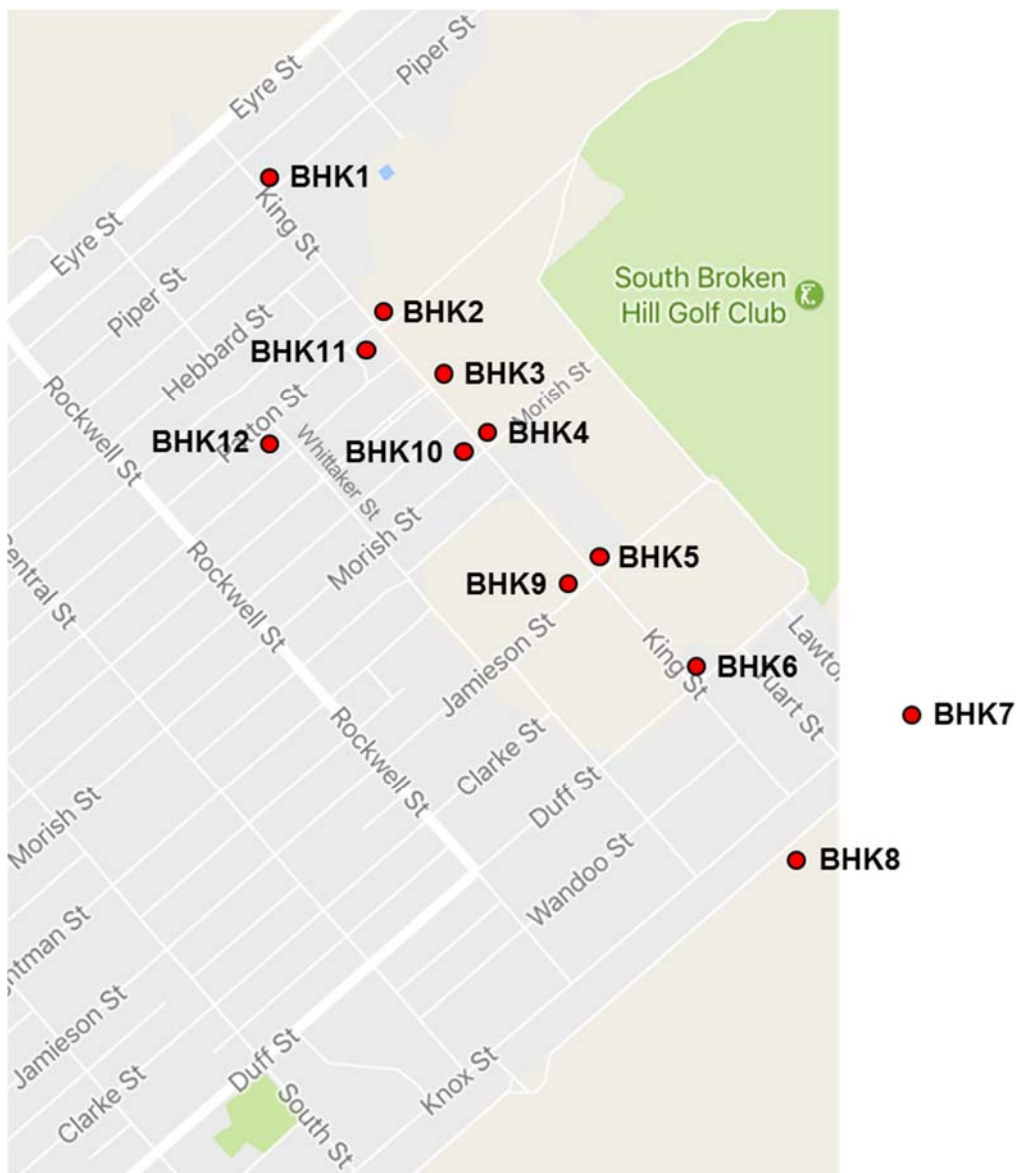


Figure 1. Broken Hill map detailing the location of soil sampling sites.

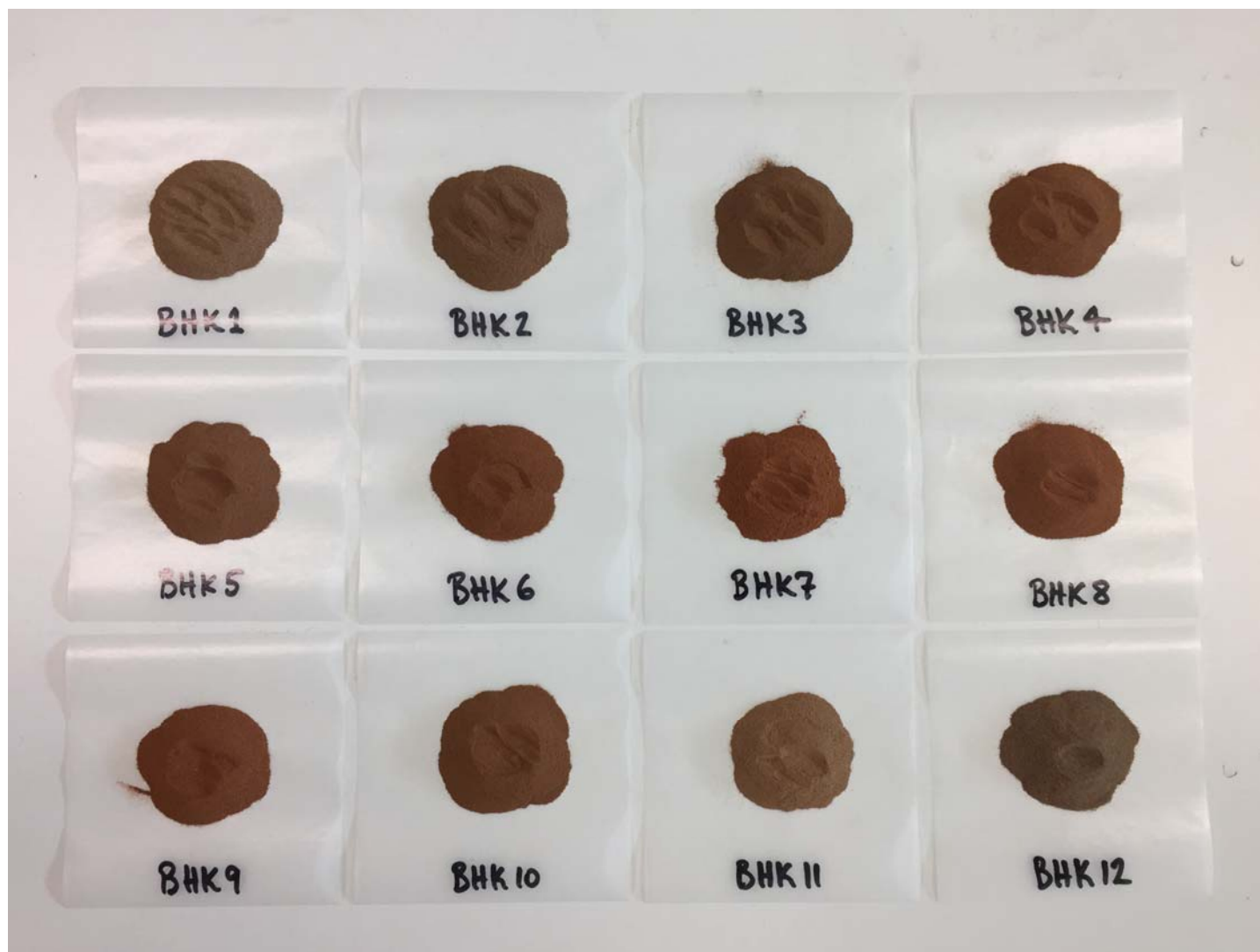


Figure 2. Broken Hill soil samples, BHK1-12 (< 250 μm soil particle size fraction).

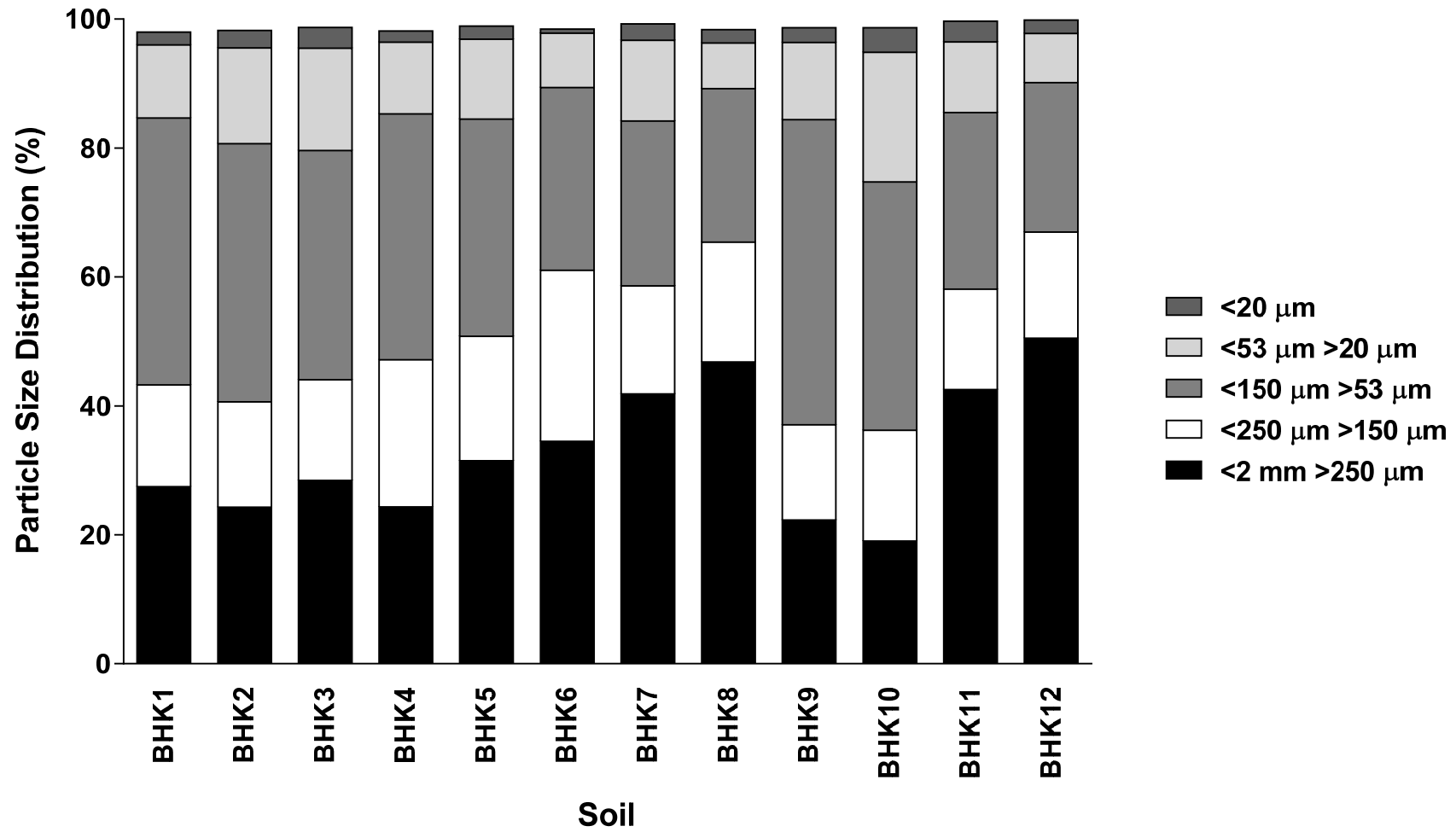


Figure 3. Particle size distribution for Broken Hill soils BHK1-12.

Metal(loid) Concentration and Speciation in BHK1-12

Lead concentration in Broken Hill soil ranged from $215 \pm 0.9 \text{ mg kg}^{-1}$ (BHK8) to $8036 \pm 651 \text{ mg kg}^{-1}$ (BHK1) in the $< 2 \text{ mm}$ soil particle size fraction (Table 1; Figure 4). Of the 12 soils sampled, only two (BHK7 and BHK8) were below the National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC) Health-based investigation level (Residential A [HILa]). The concentration of other major and minor elements are detailed in Table 1. Of note, BHK1 contained an elevated concentration of Mn ($7.1 \pm 0.8 \text{ g kg}^{-1}$) which exceed the NEPM-ASC HILa.

The concentration of Pb and other elements in the $< 250 \text{ }\mu\text{m}$ soil particle size fraction was also determined (Table 2) as this particle size fraction was utilised for bioaccessibility and relative bioavailability assays. Utilisation of the $< 250 \text{ }\mu\text{m}$ soil particle size fraction is based on the premise that this fraction adheres to the hands of young children and is available for hand-to-mouth transfer (Ruby et al., 1996; USEPA, 2007). As detailed in Figures 4 and 5, Pb was enriched in the smaller soil particle size fraction (on average by ~16%) with Pb concentrations ranging from $267 \pm 14 \text{ mg kg}^{-1}$ (BHK8) to $9930 \pm 468 \text{ mg kg}^{-1}$ (BHK1). Enrichment of other elements in the $< 250 \text{ }\mu\text{m}$ soil particle size fraction was also observed as detailed in Table 2. Notably, the concentration of Cd, Zn and Mn exceeded NEPM HILa values in BHK1. Strong relationships were observed between the concentration of Pb and Mn (Mn concentration in the $< 250 \text{ }\mu\text{m}$ fraction = $0.86 * \text{Pb concentration in the } < 250 \text{ }\mu\text{m}$ fraction + 331; $r^2 = 0.92$) or Zn (Zn concentration in the $< 250 \text{ }\mu\text{m}$ fraction = $0.72 * \text{Pb concentration in the } < 250 \text{ }\mu\text{m}$ fraction + 293; $r^2 = 0.98$) in the $< 250 \text{ }\mu\text{m}$ soil particle size fraction (Figure 6). Potentially these relationships could be utilise to ascertain the contribution of outdoor soil to indoor dust Pb concentrations.

When Pb speciation was assessed using x-ray absorption spectroscopy, Pb in BHK1-12 was present predominantly was sorbed phases to oxides and clays (45-66%). Lead was also present as plumbojarosite (6-35%), Pb phosphate (5-19%) or bound to organic matter (8-22%) (Table 3). Oxide sorbed or organic bound Pb species are often associated with redistributed Pb phases while plumbojarosite is commonly found in the oxidised zone of Pb deposits, particularly in arid regions. Lead speciation was significantly different when compared to samples collected on the northern side of the line of lode (near South Rd / Gaffney St) where Pb was present as Pb sulphate (65-91%), Pb carbonate and Pb birnessite. In BHK1-12, Fe was present predominantly as ferrihydrite (26- 68%) and associated with clay (17-41%) with smaller proportions of goethite, magnetite and pyrrhotite. Significant differences in Fe speciation was also observed with samples from the northern side of the line of lode. Differences in Pb and Fe speciation may impact on bioavailability outcomes due to differences in the solubility of Pb and Fe phases.

The concentration of Pb and other elements was also determined in the $< 150 \text{ }\mu\text{m}$ soil particle size fraction (Table 4). This was undertaken as recently the USEPA (2016) revised the particle size fraction for the assessment of Pb bioavailability (from $< 250 \text{ }\mu\text{m}$ to $< 150 \text{ }\mu\text{m}$) based upon an expanding body of evidence illustrating that dermally adhered soil is dominated by particles $< 150 \text{ }\mu\text{m}$ in size. For 5 of the 12 soils (BHK1, 5, 6, 7, 12), a significant ($p < 0.05$) increase in Pb concentration was observed in the $< 150 \text{ }\mu\text{m}$ soil particle size fraction (Figure 4) with enrichment factors ranging from 1.08 (BHK 5 and BHK 12) to 1.19 (BHK 7). Other major and minor elements were enriched in the smaller particle size fraction as detailed in Table 4.

Table 1. Concentration of major and minor elements in the < 2 mm soil particle size fraction of samples BHK1-12.

Sample	'Minor' Elements (mg kg ⁻¹)									'Major' Elements (g kg ⁻¹)					
	As	Cd	Co	Cr	Cu	Ni	P	Pb	Zn	Al	Ca	Fe	K	Mg	Mn
BHK1	44.4 ± 5.6	18.1 ± 1.7	7.8 ± 0.6	7.7 ± 1.0	97.2 ± 3.4	10.0 ± 0.6	1126 ± 85	8036 ± 651	5381 ± 314	16.0 ± 0.1	19.9 ± 1.4	22.5 ± 1.1	3.9 ± 0.2	3.8 ± 0.0	7.1 ± 0.8
BHK2	23.4 ± 0.3	8.9 ± 1.2	9.4 ± 0.5	13.7 ± 1.3	65.6 ± 2.7	13.9 ± 2.0	660 ± 45	3252 ± 208	2485 ± 27	20.6 ± 2.5	16.2 ± 0.7	28.6 ± 3.2	6.0 ± 0.1	5.0 ± 0.4	2.5 ± 0.2
BHK3	43.5 ± 3.6	18.2 ± 0.1	10.1 ± 0.2	15.7 ± 0.2	96.9 ± 0.5	16.8 ± 1.1	1135 ± 23	6128 ± 239	4814 ± 7.8	27.8 ± 0.1	24.0 ± 0.3	29.4 ± 0.6	6.7 ± 0.2	6.2 ± 0.2	4.0 ± 0.2
BHK4	12.1 ± 2.8	8.3 ± 1.8	7.0 ± 0.3	7.6 ± 0.6	41.5 ± 1.9	11.1 ± 0.1	476 ± 54	1746 ± 32	1484 ± 73	16.3 ± 1.5	15.2 ± 1.4	20.4 ± 1.6	6.4 ± 2.4	4.4 ± 0.1	1.4 ± 0.1
BHK5	20.9 ± 2.1	14.6 ± 1.0	6.8 ± 0.1	10.7 ± 0.3	50.8 ± 1.5	14.5 ± 3.1	772 ± 15	3012 ± 170	2715 ± 37	19.9 ± 2.5	17.7 ± 1.5	21.9 ± 1.7	4.9 ± 0.1	5.7 ± 0.4	1.8 ± 0.1
BHK6	4.4 ± 0.3	2.6 ± 0.1	6.0 ± 0.1	15.2 ± 0.6	24.2 ± 1.2	11.4 ± 0.5	229 ± 13	490 ± 26	440 ± 23	11.9 ± 0.1	6.7 ± 2.0	20.3 ± 1.0	4.3 ± 0.0	4.2 ± 0.7	0.6 ± 0.0
BHK7	4.7 ± 0.6	3.3 ± 1.1	9.1 ± 0.5	31.6 ± 4.7	25.7 ± 3.5	21.8 ± 6.2	121 ± 17	219 ± 33	287 ± 41	19.4 ± 1.3	1.0 ± 0.1	28.0 ± 0.5	3.6 ± 0.3	2.4 ± 0.1	0.6 ± 0.1
BHK8	5.5 ± 0.8	4.7 ± 0.5	6.0 ± 0.5	20.3 ± 2.4	23.1 ± 1.7	14.9 ± 1.9	315 ± 53	215 ± 0.9	271 ± 53	16.5 ± 0.5	21.5 ± 2.0	26.3 ± 6.0	4.9 ± 0.1	2.9 ± 0.0	0.8 ± 0.2
BHK9	6.0 ± 0.4	3.8 ± 0.1	5.8 ± 0.1	16.8 ± 0.6	28.7 ± 1.3	12.5 ± 0.6	214 ± 8.7	427 ± 25	341 ± 11	14.8 ± 0.2	7.5 ± 0.1	21.5 ± 1.7	4.4 ± 0.2	3.1 ± 0.0	0.9 ± 0.1
BHK10	11.6 ± 1.4	5.1 ± 0.3	11.0 ± 0.4	32.7 ± 3.6	68.9 ± 2.8	23.4 ± 0.9	675 ± 15	1578 ± 51	1480 ± 26	29.4 ± 0.0	14.3 ± 0.1	34.0 ± 0.4	7.3 ± 0.0	10.5 ± 0.3	1.4 ± 0.1
BHK11	10.6 ± 0.7	4.9 ± 0.2	6.6 ± 0.1	19.2 ± 3.5	27.3 ± 0.2	15.3 ± 2.0	320 ± 4.5	773 ± 58	626 ± 30	14.0 ± 0.0	47.2 ± 1.7	18.1 ± 0.2	3.5 ± 0.1	3.9 ± 0.1	1.0 ± 0.2
BHK12	12.4 ± 1.0	5.0 ± 0.7	9.4 ± 0.6	40.8 ± 2.5	40.8 ± 3.2	24.3 ± 13	366 ± 22	969 ± 1.0	774 ± 15	12.6 ± 0.0	9.6 ± 0.7	29.6 ± 0.7	6.1 ± 0.1	4.0 ± 0.0	1.5 ± 0.5

Table 2. Concentration of major and minor elements in the < 250 µm soil particle size fraction of samples BHK1-12.

Sample	'Minor' Elements (mg kg ⁻¹)									'Major' Elements (g kg ⁻¹)					
	As	Cd	Co	Cr	Cu	Ni	P	Pb	Zn	Al	Ca	Fe	K	Mg	Mn
BHK1	63.1 ± 4.9	22.2 ± 1.3	7.8 ± 0.1	8.5 ± 0.5	111 ± 1.4	9.7 ± 0.2	1422 ± 5.5	9930 ± 468	7081 ± 221	17.7 ± 0.6	23.4 ± 0.4	23.7 ± 1.3	4.0 ± 0.0	3.7 ± 0.1	10.2 ± 0.3
BHK2	34.7 ± 3.3	9.3 ± 0.3	11.0 ± 0.8	19.1 ± 2.3	81.0 ± 3.1	15.2 ± 0.7	808 ± 1.0	3670 ± 178	3140 ± 139	23.2 ± 0.8	16.7 ± 0.9	30.6 ± 1.5	7.0 ± 0.2	5.6 ± 0.3	3.5 ± 0.2
BHK3	48.4 ± 2.1	18.5 ± 1.4	10.6 ± 0.9	16.9 ± 3.3	97.1 ± 12	17.8 ± 1.8	1184 ± 105	6416 ± 453	5236 ± 514	28.7 ± 2.6	27.4 ± 2.2	31.3 ± 3.0	7.0 ± 0.4	6.5 ± 0.5	4.5 ± 0.4
BHK4	16.0 ± 1.1	8.2 ± 0.4	7.6 ± 0.7	11.4 ± 1.2	46.0 ± 3.7	13.0 ± 0.8	535 ± 9.2	2107 ± 66	1844 ± 115	19.6 ± 1.7	16.3 ± 0.7	24.1 ± 1.6	5.0 ± 0.1	5.1 ± 0.3	1.8 ± 0.1
BHK5	25.9 ± 0.7	16.6 ± 0.4	7.2 ± 0.7	11.2 ± 2.4	59.0 ± 5.4	14.5 ± 0.4	1015 ± 14	3536 ± 51	3202 ± 231	20.5 ± 0.9	18.2 ± 0.2	24.5 ± 0.3	5.2 ± 0.3	6.2 ± 0.0	2.3 ± 0.2
BHK6	6.3 ± 0.8	3.2 ± 0.2	7.2 ± 0.1	18.3 ± 0.1	30.4 ± 1.4	13.1 ± 0.1	260 ± 28	582 ± 12	540 ± 13	13.5 ± 0.4	6.1 ± 0.3	23.7 ± 0.6	4.8 ± 0.2	4.2 ± 0.2	0.7 ± 0.0
BHK7	6.4 ± 0.1	3.7 ± 0.1	10.7 ± 0.1	42.8 ± 9.2	29.9 ± 0.6	25.0 ± 4.4	176 ± 3.2	316 ± 1.0	389 ± 2.7	24.3 ± 0.2	1.4 ± 0.0	33.1 ± 1.0	4.4 ± 0.0	2.9 ± 0.0	0.7 ± 0.0
BHK8	6.5 ± 0.5	5.4 ± 0.4	7.0 ± 0.2	22.5 ± 0.2	26.6 ± 0.6	14.8 ± 0.5	357 ± 42	267 ± 14	456 ± 78	17.2 ± 0.1	21.7 ± 0.6	24.7 ± 0.3	5.5 ± 0.1	2.9 ± 0.0	1.5 ± 0.4
BHK9	6.8 ± 1.1	3.9 ± 0.1	6.8 ± 0.2	19.7 ± 0.2	33.0 ± 0.5	13.8 ± 0.4	186 ± 1.4	452 ± 18	369 ± 11	16.5 ± 0.3	8.0 ± 0.2	24.5 ± 0.2	4.9 ± 0.1	3.3 ± 0.1	1.0 ± 0.0
BHK10	14.7 ± 0.4	5.7 ± 0.9	11.8 ± 0.3	35.8 ± 2.9	73.5 ± 0.1	26.6 ± 2.3	516 ± 9.6	1572 ± 16	1722 ± 26	21.4 ± 0.4	10.3 ± 0.1	35.8 ± 0.6	5.7 ± 0.0	7.6 ± 0.2	1.6 ± 0.0
BHK11	15.4 ± 0.1	5.7 ± 0.1	8.0 ± 0.1	23.7 ± 2.0	38.7 ± 2.4	18.9 ± 2.1	419 ± 32	1097 ± 15	948 ± 25	17.0 ± 0.1	42.9 ± 1.0	23.7 ± 0.4	4.3 ± 0.1	4.6 ± 0.0	1.5 ± 0.0
BHK12	19.2 ± 3.3	6.2 ± 0.1	11.8 ± 1.3	31.5 ± 3.6	45.7 ± 2.9	18.4 ± 0.9	477 ± 16	1312 ± 42	1201 ± 78	16.0 ± 1.0	12.2 ± 0.1	38.0 ± 3.4	7.4 ± 0.2	5.0 ± 0.1	1.8 ± 0.2

Table 3. Lead and iron speciation in Broken Hill soil (< 250 µm soil particle size fraction).

Sample	Pb Speciation, Weighted Percentage (%)							Fe Speciation, Weighted Percentage (%)						
	Mineral sorbed Pb*	Organic bound Pb	Plumbojarosite	Pb phosphate‡	Pb sulphate	Pb carbonate	Pb Birnessite	Ferrihydrite	Goethite	Magnetite	Pyrrhotite	Lepidocrocite	Organic bound Fe	Clay
BHK-1	62	11	13	14	-	-	-	35	15	6	25	-	-	20
BHK-2	66	9	10	16	-	-	-	39	10	8	18	-	-	25
BHK-3	63	8	10	19	-	-	-	24	6	24	10	-	-	37
BHK-4	64	11	6	19	-	-	-	45	11	10	9	-	-	26
BHK-5	58	13	24	5	-	-	-	31	11	11	15	-	-	31
BHK-6	49	16	23	12	-	-	-	30	16	12	5	-	-	36
BHK-7	51	8	35	6	-	-	-	26	13	11	18	-	-	32
BHK-8	49	8	34	8	-	-	-	34	13	9	11	-	-	34
BHK-9	47	21	22	10	-	-	-	36	12	11	10	-	-	31
BHK-10	57	11	26	6	-	-	-	33	15	5	6	-	-	41
BHK-11	45	21	23	12	-	-	-	34	13	0	7	-	-	47
BHK-12	51	22	18	9	-	-	-	68	7	4	4	-	-	17
A1†	-	-	-	-	91	9	-	36	11	-	-	15	24	14
B1†	-	-	-	3	65	-	32	32	15	-	-	19	20	14

*Pb sorbed to oxides and clays

‡Sum of pyromorphite and Pb₃(PO₄)₂.

†Samples A1 and B1 were collected from the northern side of the line of lode (near South Rd / Gaffney St).

Table 4. Concentration of major and minor elements in the < 150 µm soil particle size fraction of samples BHK1-12.

Sample	'Minor' Elements (mg kg ⁻¹)									'Major' Elements (g kg ⁻¹)					
	As	Cd	Co	Cr	Cu	Ni	P	Pb	Zn	Al	Ca	Fe	K	Mg	Mn
BHK1	84.8 ± 2.0	24.4 ± 0.2	9.1 ± 0.3	9.1 ± 1.3	139 ± 12	11.0 ± 0.5	1682 ± 49	11744 ± 10	8145 ± 324	20.2 ± 0.2	27.0 ± 0.2	26.9 ± 0.0	4.2 ± 0.0	4.0 ± 0.0	12.1 ± 0.1
BHK2	34.3 ± 4.6	9.5 ± 0.1	10.8 ± 0.6	18.2 ± 0.9	81.5 ± 5.7	15.7 ± 1.3	876 ± 17	3998 ± 88	3278 ± 157	24.7 ± 0.2	18.3 ± 0.5	32.2 ± 0.9	7.2 ± 0.2	5.8 ± 0.2	3.9 ± 0.1
BHK3	52.0 ± 1.3	17.6 ± 0.8	10.3 ± 0.1	16.7 ± 0.6	100 ± 1.4	18.2 ± 0.1	1160 ± 30	6533 ± 118	5240 ± 57	25.8 ± 1.5	25.8 ± 0.7	29.6 ± 0.6	6.7 ± 0.1	6.1 ± 0.2	4.4 ± 0.2
BHK4	22.6 ± 1.9	8.7 ± 0.3	8.6 ± 0.1	12.8 ± 0.3	53.6 ± 0.5	13.7 ± 1.2	734 ± 28	2250 ± 102	2127 ± 6.5	21.2 ± 1.3	17.2 ± 0.7	25.3 ± 1.5	5.8 ± 0.2	5.3 ± 0.3	2.4 ± 0.0
BHK5	29.9 ± 3.1	18.8 ± 0.1	8.0 ± 0.2	14.5 ± 0.4	67.6 ± 1.3	15.8 ± 0.7	1092 ± 1.4	3836 ± 4.5	3833 ± 92	24.6 ± 0.4	20.9 ± 0.0	27.2 ± 0.3	5.8 ± 0.1	6.9 ± 0.0	3.0 ± 0.0
BHK6	7.1 ± 0.2	3.6 ± 0.1	7.8 ± 0.1	21.4 ± 0.3	35.2 ± 0.5	15.0 ± 0.4	276 ± 0.5	679 ± 18	631 ± 2.3	15.8 ± 0.8	6.9 ± 0.2	26.8 ± 0.1	5.3 ± 0.1	4.7 ± 0.1	0.8 ± 0.0
BHK7	7.3 ± 0.5	3.8 ± 0.1	11.2 ± 0.4	35.9 ± 0.5	33.5 ± 2.6	21.7 ± 0.9	196 ± 2.1	375 ± 5.3	452 ± 10	25.9 ± 0.3	1.5 ± 0.0	34.2 ± 0.4	4.8 ± 0.0	3.2 ± 0.0	0.8 ± 0.0
BHK8	6.9 ± 0.1	5.0 ± 0.1	7.5 ± 0.1	25.9 ± 0.6	30.0 ± 0.7	16.2 ± 0.7	304 ± 7.5	298 ± 5.6	400 ± 7.9	19.3 ± 0.2	23.7 ± 0.4	28.3 ± 0.9	5.9 ± 0.1	3.1 ± 0.0	1.2 ± 0.0
BHK9	6.4 ± 0.2	3.7 ± 0.3	6.5 ± 0.6	18.5 ± 0.5	32.8 ± 2.7	13.4 ± 0.4	197 ± 9.3	483 ± 16	376 ± 4.9	15.2 ± 0.2	7.9 ± 0.3	23.7 ± 1.0	4.7 ± 0.2	3.3 ± 0.2	0.8 ± 0.0
BHK10	17.8 ± 3.4	4.8 ± 0.3	12.1 ± 0.8	35.7 ± 2.2	74.7 ± 4.5	25.9 ± 0.7	530 ± 4.3	1572 ± 18	1759 ± 121	21.6 ± 0.8	10.7 ± 0.3	35.7 ± 1.8	6.1 ± 0.0	7.6 ± 0.2	1.7 ± 0.1
BHK11	18.3 ± 1.6	6.1 ± 0.2	8.2 ± 0.1	22.1 ± 0.9	39.5 ± 3.1	16.4 ± 0.3	443 ± 14	1180 ± 12	1051 ± 14	17.4 ± 0.6	42.2 ± 1.1	23.8 ± 0.0	4.4 ± 0.2	4.5 ± 0.1	1.9 ± 0.1
BHK12	22.2 ± 0.4	6.9 ± 0.1	12.2 ± 0.1	32.5 ± 0.2	50.4 ± 0.4	19.8 ± 0.2	514 ± 7.7	1419 ± 4.7	1343 ± 11	17.2 ± 0.5	12.9 ± 0.1	40.3 ± 0.3	7.8 ± 0.1	5.2 ± 0.0	2.1 ± 0.2

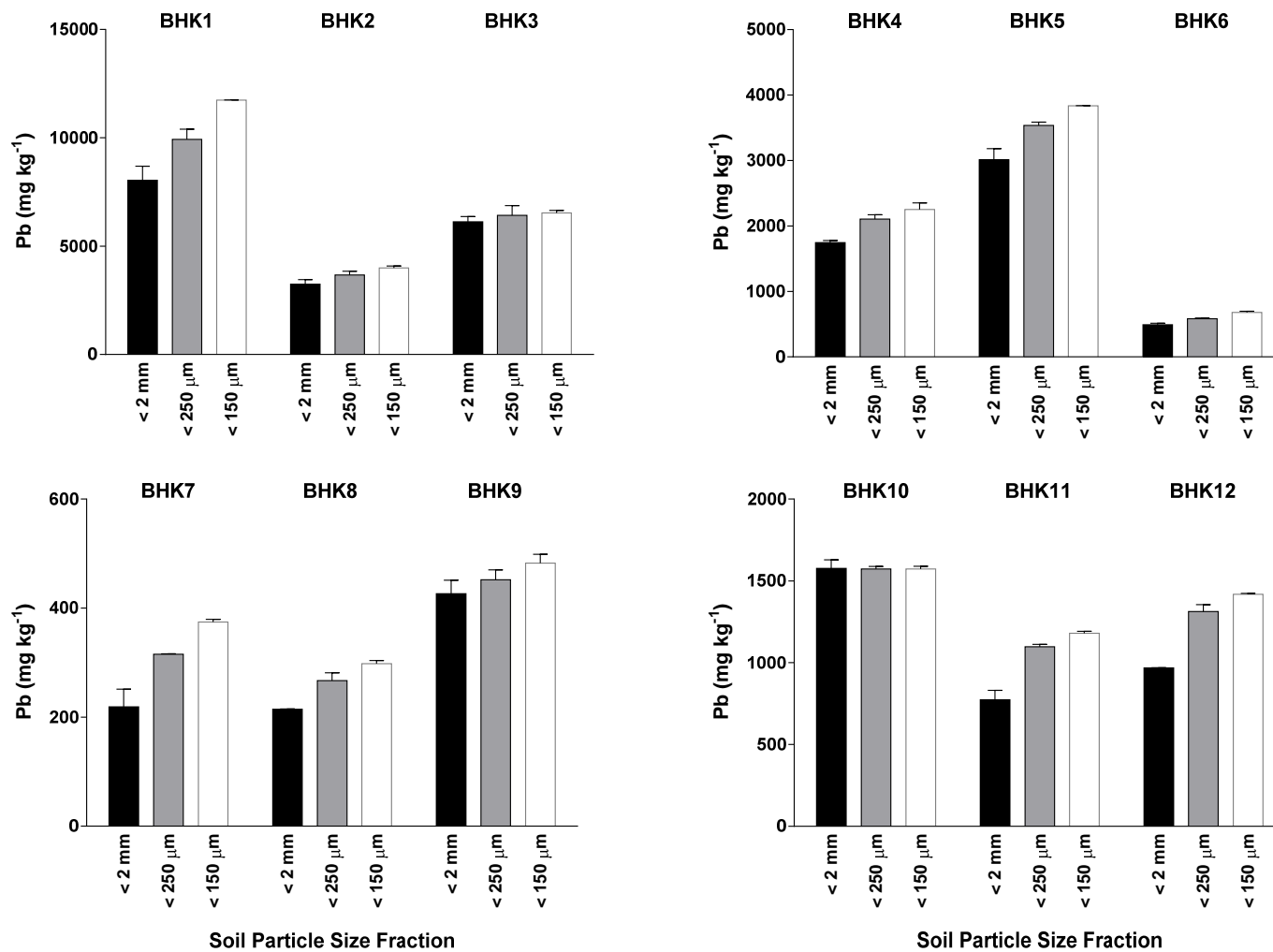


Figure 4. Comparison of Pb concentration in Broken Hill soil particle size fractions (< 2 mm, < 250 μm and < 150 μm).

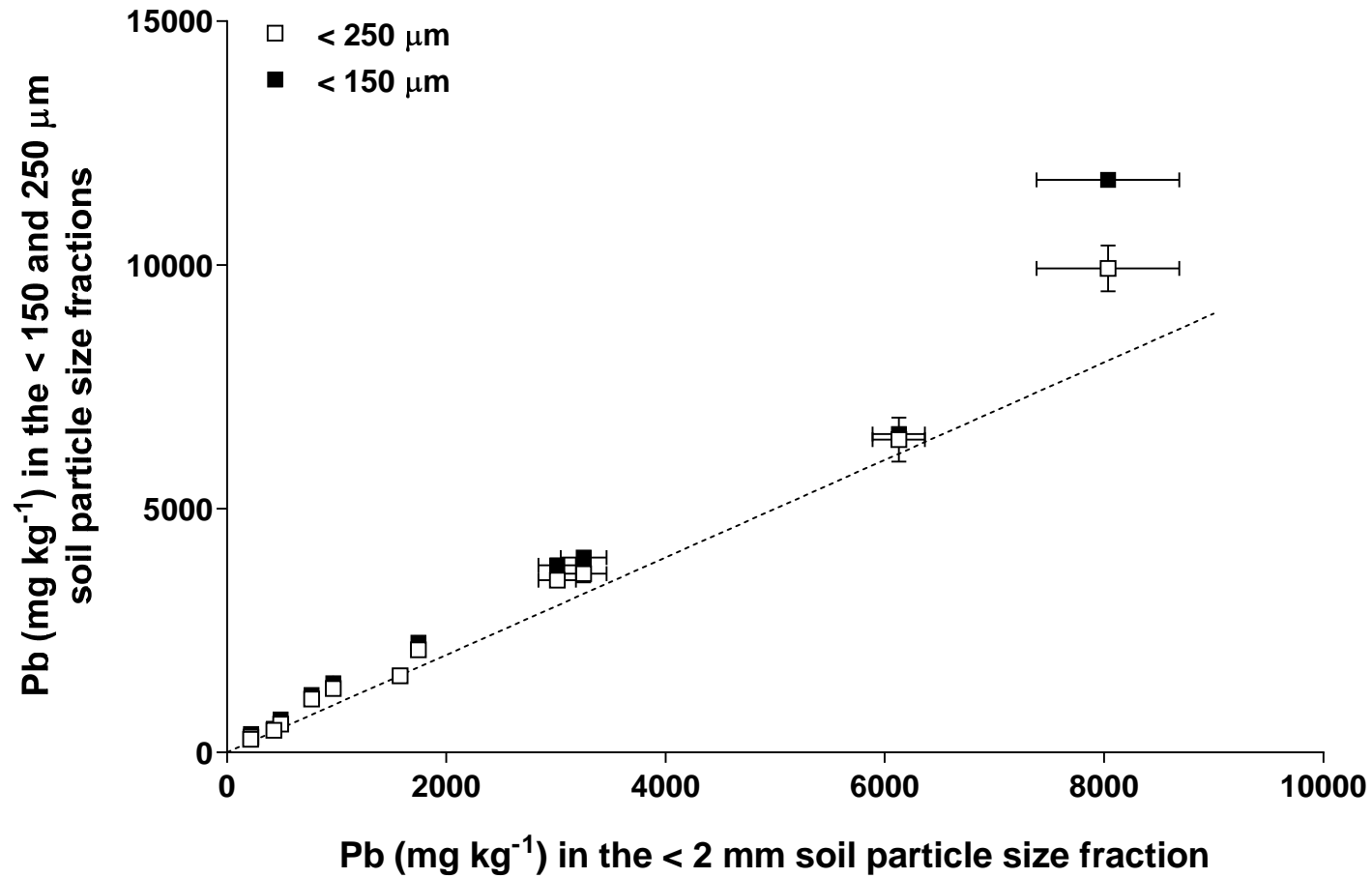


Figure 5. Relationship between Pb concentration in the < 2 mm soil particle size fraction and the < 250 μm (□) and < 150 μm (■) soil particle size fractions. The dotted line represents a 1:1 relationship.

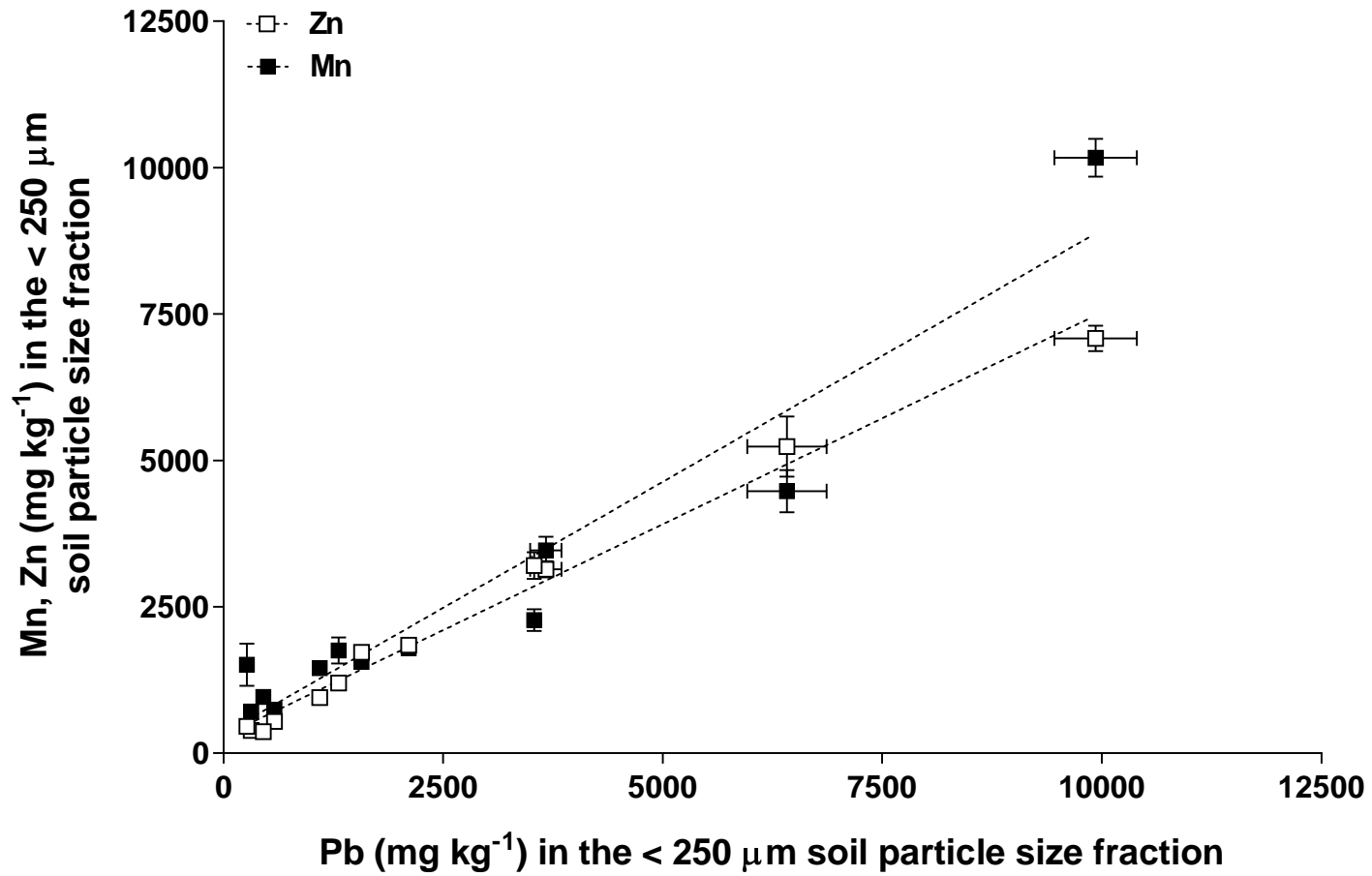


Figure 6. Relationship between Pb concentration in the < 250 μm soil particle size fraction and the concentration of Mn (■) and Zn (□).

Assessment of Lead Bioaccessibility

Lead bioaccessibility in Broken Hill soil was assessed using the Solubility Bioaccessibility Research Consortium (SBRC) assay incorporating both gastric (SBRC-G) and intestinal phases (SBRC-I). Although gastric phase extraction has been recommended by the USEPA for estimating Pb relative bioavailability, there are issues using this methodology when Pb mineralogy is not the controlling parameter for Pb dissolution (a caveat detailed in USEPA, 2012). In particular, the mineralogy of the environmental matrix (e.g. Fe concentration and speciation) may significantly influence dissolution-precipitation events that controls Pb relative bioavailability. As a consequence both gastric and intestinal phases of the SBRC assay were utilised to assess Pb bioaccessibility in both the < 250 µm and < 150 µm soil particle size fractions.

As detailed in Figure 7, Pb bioaccessibility in the < 250 µm soil particle size fraction was significantly higher when assessed using gastric phase extraction compared to the intestinal phase. With the exception of BHK4 ($42.7 \pm 7.1\%$), BHK8 ($41.5 \pm 2.6\%$) and BHK9 ($54.6 \pm 2.5\%$), Pb bioaccessibility following gastric phase extraction was > 60% (ranging from 61.7% [BHK12] to 79.5% [BHK10]). However, when gastric phase conditions were transitioned into the intestinal phase, Pb bioaccessibility significantly decreased (by 2.1-21.8 fold) with values ranging from $1.9 \pm 0.2\%$ (BHK8) to $25.3 \pm 1.1\%$ (BHK12). Following dissolution in the low pH environment of the SBRC gastric phase, Pb may precipitate (or co-precipitate with Fe) once assay conditions are modified to the intestinal phase (i.e. addition of bile / pancreatin and pH neutralisation). Lead precipitation when the pH is increased to 7.0 ± 0.2 is attributed to the exceedance of Pb solubility constants and with the association of Pb with Fe oxide phases (Smith et al., 2011). With the increase in pH from gastric to intestinal phases, Fe may also become oversaturated and precipitate, resulting in a decrease in soluble Pb as a consequence of Pb sorption to Fe (oxy)hydroxides (Mercer and Tobiasson, 2008). The largest decrease in gastric to intestinal phase Pb bioaccessibility was observed for BHK7, 8 and 9, soils with the lowest total Pb concentration.

Similar trends in Pb bioaccessibility were observed for the < 150 µm soil particle size fraction. Gastric phase Pb bioaccessibility ranged from $37.3 \pm 1.0\%$ (BHK8) to $72.8 \pm 1.4\%$ (BHK1) while intestinal phase values were 2.9 (BHK4, 12) to 20.7 fold (BHK8) lower (ranging from $1.8 \pm 0.1\%$ [BHK8] to $16.6 \pm 1.5\%$ [BHK2]). While some variability in Pb bioaccessibility was observed between the < 250 µm and < 150 µm soil particle size fractions, gastric phase values were not significantly different ($p > 0.05$) for 6 of the 12 soils (BHK1, 2, 3, 4, 5, 8) while the remaining values varied by < 20% between particle size fractions. Lower intestinal phase Pb bioaccessibility values were observed for the < 150 µm soil particle size fraction compared to the < 250 µm soil fraction. Presumably differences in the concentration of elements such as Fe and P influenced the outcomes of intestinal phase assessments.

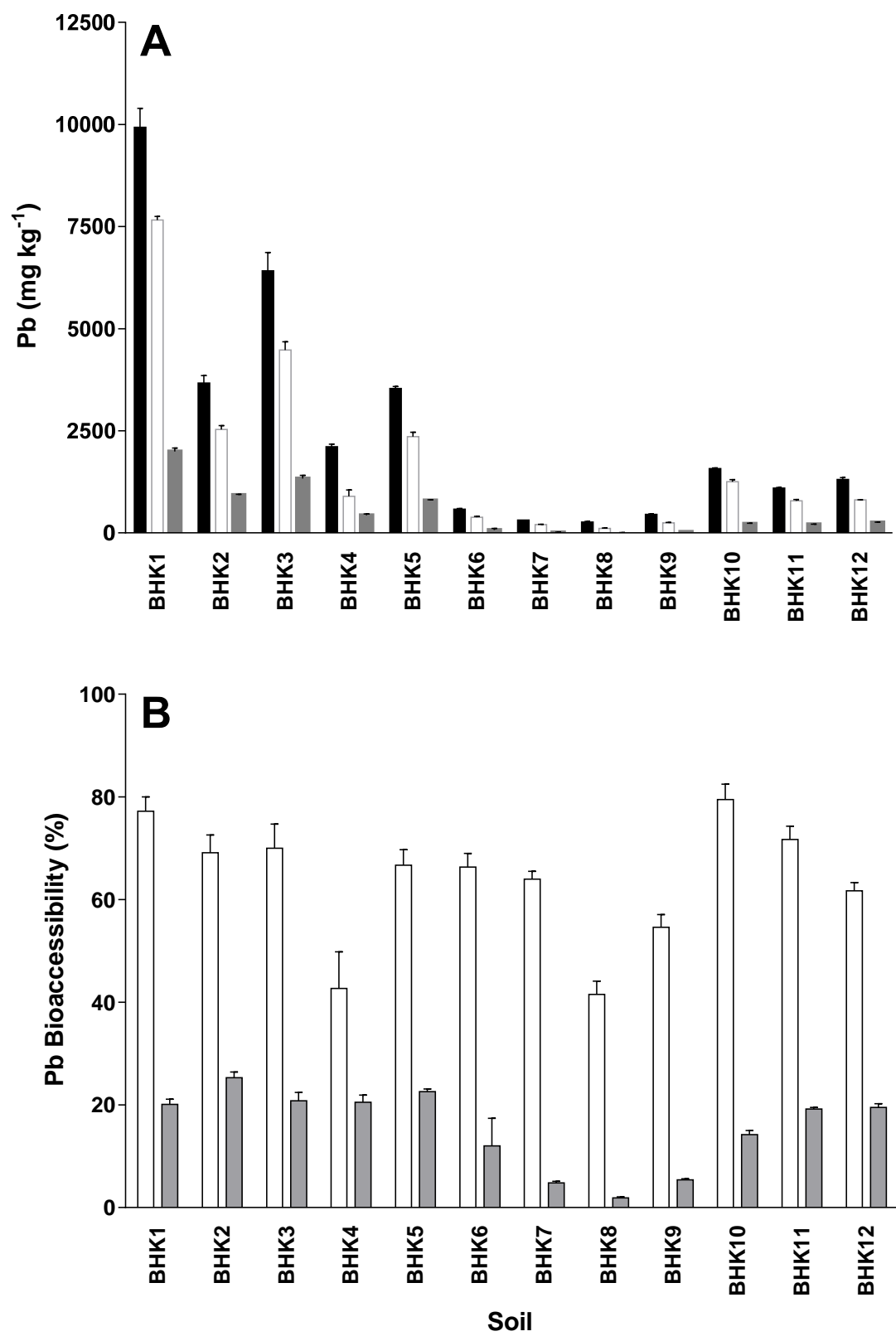


Figure 7. Lead bioaccessibility in the < 250 μm soil particle size fraction of BHK1-12 determined using gastric (□) and intestinal (■) phases of the SBRC assay. Panel A shows the total (■) and bioaccessible Pb concentration in samples BHK1-12 while panel B shows percentage bioaccessibility for gastric and intestinal phase extractions.

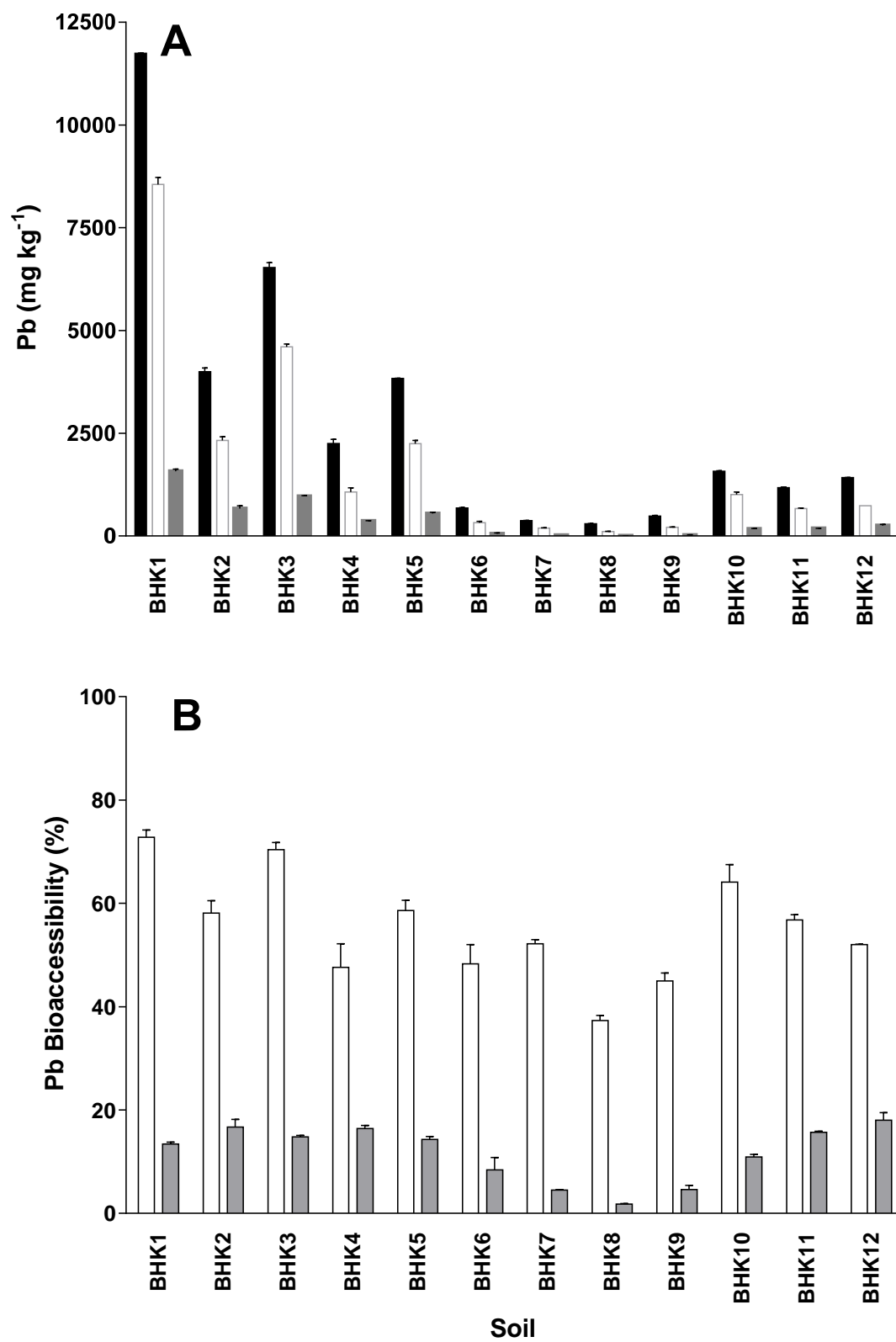


Figure 8. Lead bioaccessibility in the < 150 μm soil particle size fraction of BHK1-12 determined using gastric (□) and intestinal (■) phases of the SBRC assay. Panel A shows the total (■) and bioaccessible Pb concentration in samples BHK1-12 while panel B shows percentage bioaccessibility for gastric and intestinal phase extractions.

Assessment of Lead Relative Bioavailability

Lead relative bioavailability in Broken Hill soil (n = 10) was assessed using the USEPA's Integrated Systems Toxicology Division mouse model protocol (IRP-NHEERL-RTP/ISTD). This methodology included a 10 day exposure period (i.e. multiple dosing scheme) by incorporating Pb-contaminated soil or Pb acetate into standard mouse chow (1% w/w). A point estimate approach was utilised to determine Pb relative bioavailability by comparing the accumulation of Pb in target organs (liver, kidney and femur) following exposure to Pb-contaminated soil (1% w/w) or Pb acetate in AIN93G mouse chow (15 mg kg⁻¹; reference dose).

Figure 9 shows the range in percentage Pb relative bioavailability for Broken Hill soil while Table 5 shows the minimum, maximum and mean Pb relative bioavailability (including lower and upper 95th confidence intervals of the mean) for individual soils and the complete dataset. Mean Pb relative bioavailability in Broken Hill soil ranged from 6.8 ± 1.2% for BHK3 to 44.8 ± 5.1% for BHK9, however, for 8 of the 10 soils analysed, mean Pb relative bioavailability was < 20%. Higher Pb relative bioavailability was observed in BHK7 (33.3 ± 8.0%) and BHK9 (44.8 ± 5.1%), soils which presented 'low' total Pb concentrations (316 and 452 mg kg⁻¹ respectively). While it is known that a variety of physico-chemical properties may influence Pb relative bioavailability (e.g. particle size, other major and minor elements in the soil), the particle size distribution in BHK7 and 9 (Figure 3) was similar to other Broken Hill soils. Although some variability was observed in the molar ratio of major and minor elements to Pb in Broken Hill soil, differences between BHK7, BHK9 and other soils (e.g. elevated molar ratios of potassium and manganese to Pb) do not explained the higher Pb relative bioavailability measured in these soils. Conceivably, Pb speciation, or the proportion of Pb species, in BHK7 and 9 may be different to the other soils that were assessed which may influence Pb relative bioavailability outcomes. Solid phase Pb speciation data is currently being obtained (using X-ray Absorption Spectroscopy) through collaborators at the USEPA; it is envisaged that this data will be available for interpretation at the start of 2018.

Although additional Pb relative bioavailability studies using Broken Hill were unable to be sourced from the literature, *in vivo* assessment of mine impacted material has been undertaken for other locations worldwide (predominantly the US). As detailed in Table 5, Pb relative bioavailability, using a variety of animal models, is variable ranging from 1-105%.

Table 5. *In vivo* assessment of Pb relative bioavailability in mine impacted material.

Substrate	Animal Model	Pb Relative Bioavailability	Reference
Mine Wastes	Rat	0.6-13.3%	Freeman et al. (1992)
Mine Wastes	Rat	0.5-2.3%	Freeman et al. (1993)
Mine Wastes	Rabbit	9 ± 4%	Ruby et al. (1993)
Smelter soil	Swine	38-45%	Casteel et al. (1997)
Bunker Hill soil	Human	26.2 ± 8.1	Maddaloni et al. (1998)
Soil-like material	Swine	1-105%	Casteel et al. (2006)
Soil	Swine	17-63	Marschner et al. (2006)

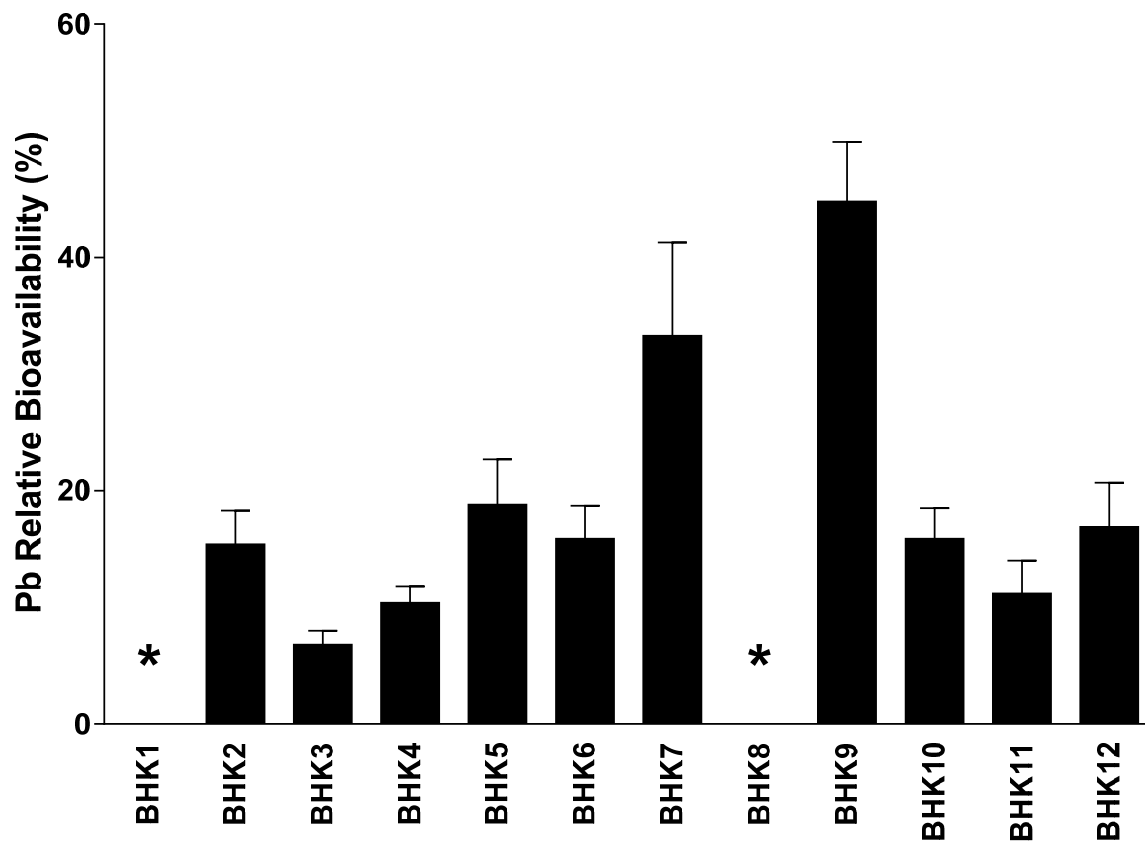


Figure 9. Percentage Pb relative bioavailability in Broken Hill soil determined using an in vivo mouse model. Bars represent the mean and standard deviation of triplicate (BHK3, 4, 6, 9, 10, 11) or quadruplicate (BHK2, 5, 7, 12) analysis. Lead relative bioavailability assessment was not performed on samples BHK1 and 8 (marked with an asterisks) as the Pb concentration in the <250 μm particle size fraction was either too high (i.e. $\sim 10,000 \text{ mg kg}^{-1}$ for BHK1) or below the NEPM HILa value (BHK8).

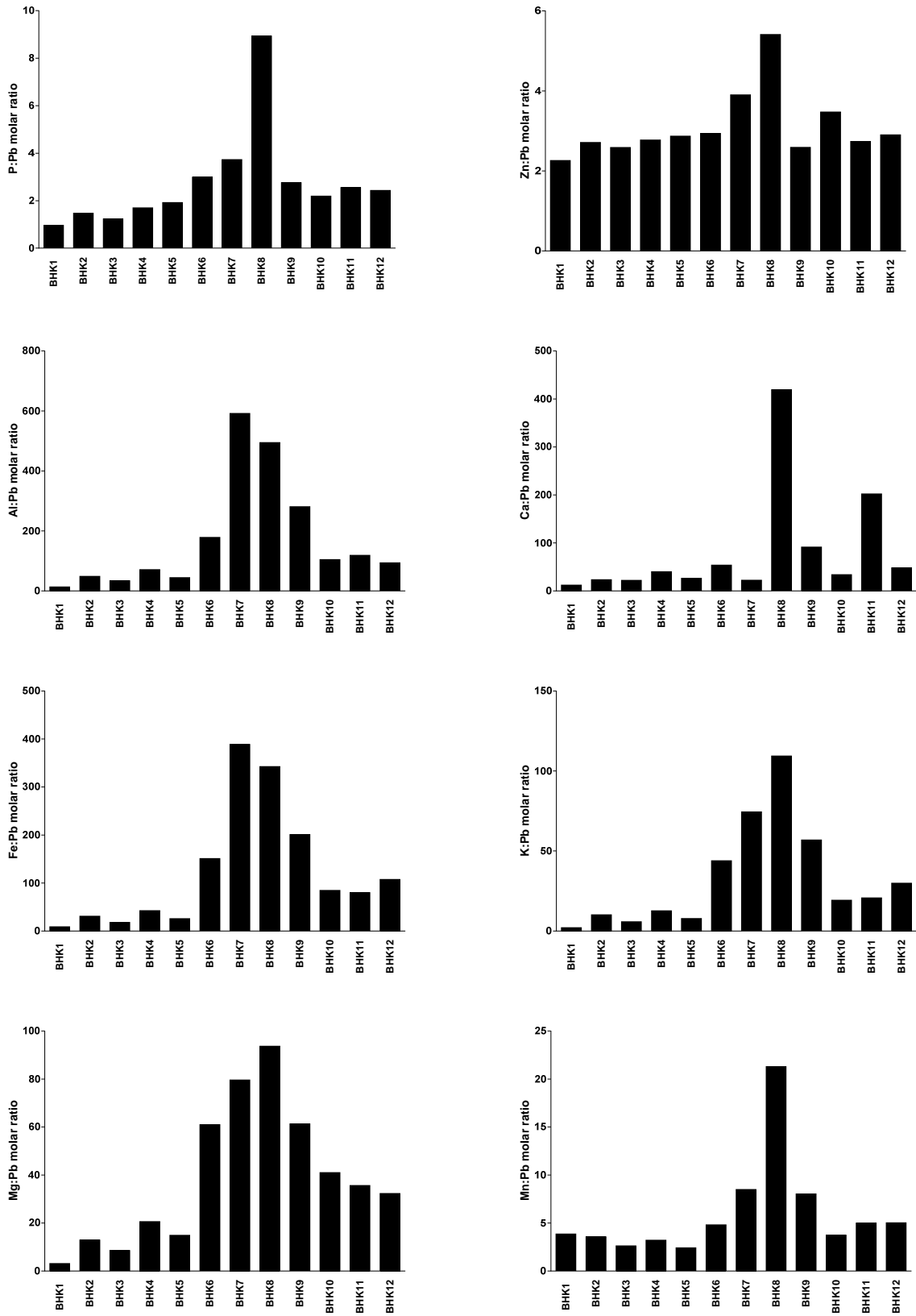


Figure 10. Molar ratio of major and minor elements to Pb in Broken Hill soil.

Table 6. Total concentration and relative bioavailability of Pb in the < 250 μm soil particle size fraction of samples BHK1-12.

Sample	Pb (mg kg^{-1})	Pb Relative Bioavailability (%)				
		Min.	Max.	Mean	Lower 95% CI of mean	Upper 95% CI of mean
BHK1	9930 \pm 468	-	-	-	-	-
BHK2	3670 \pm 178	10.2	21.4	15.4	13.9	17.0
BHK3	6416 \pm 453	5.3	8.8	6.8	5.9	7.7
BHK4	2107 \pm 66	8.8	12.4	10.4	9.3	11.5
BHK5	3536 \pm 51	12.2	25.8	18.8	16.7	20.5
BHK6	582 \pm 12	12.5	20.9	15.9	13.7	18.1
BHK7	316 \pm 1.0	19.3	46.3	33.3	29.1	37.6
BHK8	267 \pm 14	-	-	-	-	-
BHK9	452 \pm 18	38.2	54.0	44.8	40.9	48.8
BHK10	1572 \pm 16	12.1	19.9	15.9	13.8	17.9
BHK11	1097 \pm 15	7.2	15.5	11.2	9.1	13.4
BHK12	1312 \pm 42	10.5	25.1	16.9	14.9	19.0
All soils	316-6416	5.3	54.0	19.5	17.4	21.5

Comparison of Lead Relative bioavailability and Lead Bioaccessibility

The determination of Pb relative bioavailability using in vivo assays is time consuming, laborious and expensive. However, in vitro assays, that simulate gastrointestinal conditions, have the potential to act as a surrogate assay for predicting Pb relative bioavailability that is rapid and inexpensive. Currently, the USEPA prescribe USEPA method 9200 (the SBRC gastric phase) for the assessment of Pb bioaccessibility to predict Pb relative bioavailability. However, in recent years, a number of researchers have determined that the use of the SBRC gastric phase may provide a conservative estimate of Pb relative bioavailability as the assay does not account for the behaviour of Pb and other elements under intestinal phase conditions where elements are absorbed. As a consequence, this study compared in vivo and in vitro data (gastric and intestinal phases) to determine the capacity of bioaccessibility assays for predicting Pb relative bioavailability in Broken Hill soil.

As detailed in Figure 11, poor relationships were observed when Pb relative bioavailability and Pb bioaccessibility were compared. When Pb bioaccessibility was assessed using the gastric phase of the SBRC assay, values were up to 10-fold higher (1.2-10.3) compared to the corresponding Pb relative bioavailability value (Table 7). Although a poor relationship was also observed when the complete data set of SBRC intestinal phase values were compared to Pb relative bioavailability (Figure 11), this in part, was due to the narrow range of Pb relative bioavailability values in Broken Hill soil. For 4 soils (BHK 5, 6, 10, 12), there was no significant difference ($p > 0.05$) between SBRC intestinal phase values and Pb relative bioavailability while for BHK2, 3, 4 and 11, in vitro assessment over-predicted Pb relative bioavailability by 1.6-3.1 fold. Where elevated Pb relative bioavailability was observed (BHK7, 9), assessment of Pb bioaccessibility using the SBRC intestinal phase, significantly underestimated Pb relative bioavailability.

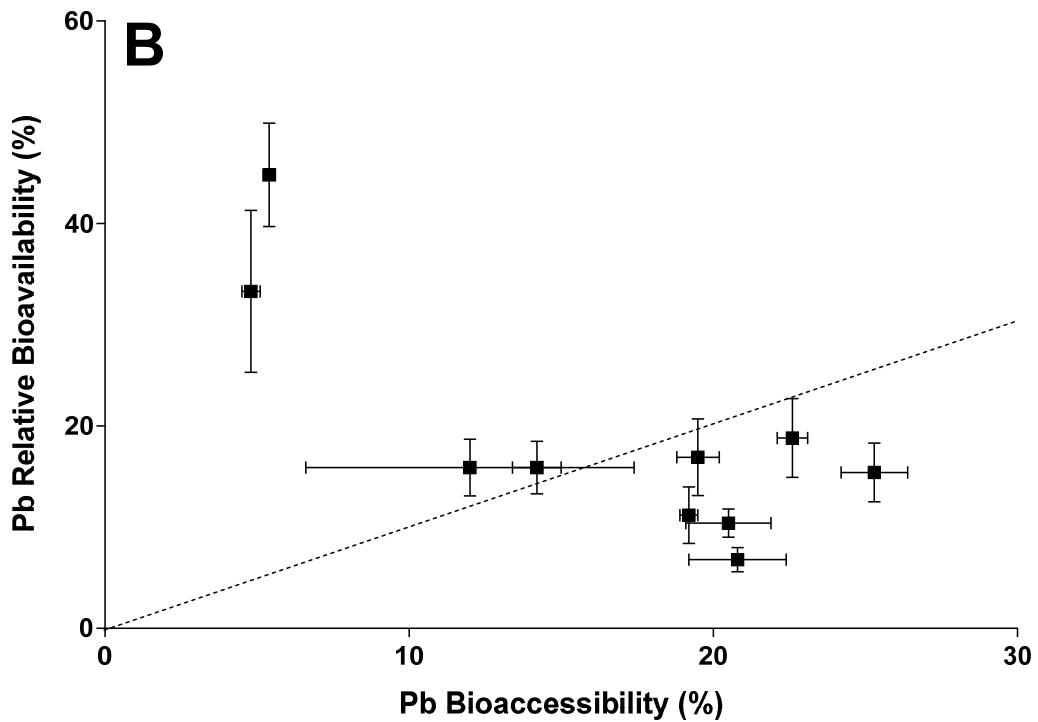
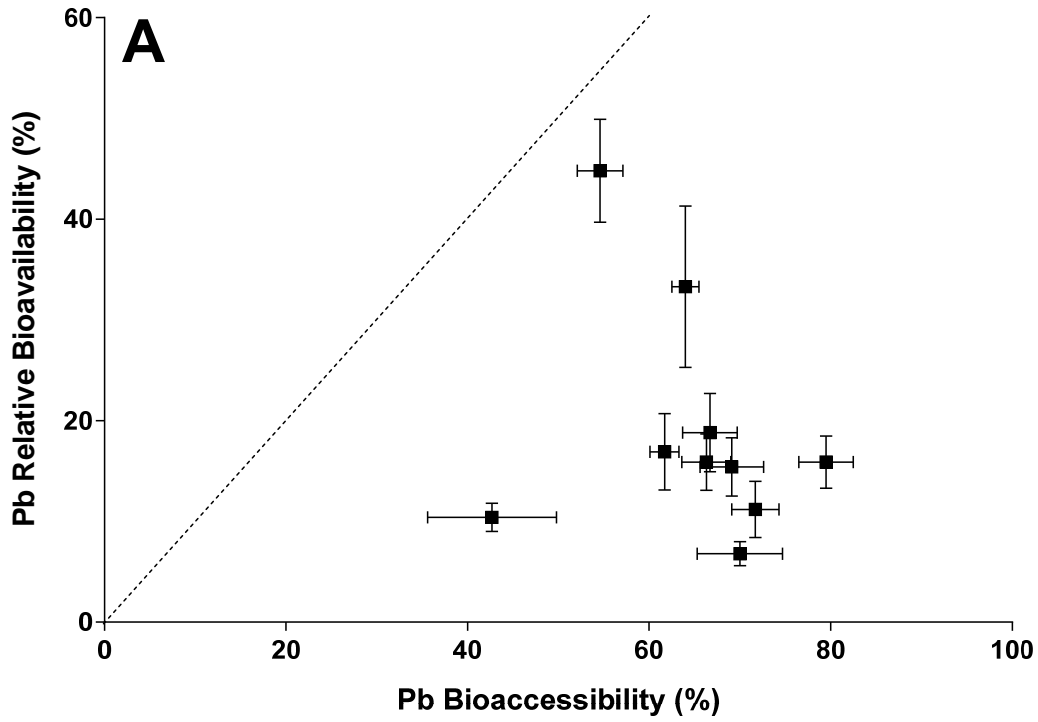


Figure 11. Relationship between Pb relative bioavailability, determined using an in vivo mouse model, and Pb bioaccessibility, determined using gastric (A) and intestinal (B) phase extraction of the SBRC assay. The dotted line represents a 1:1 relationship between Pb relative bioavailability and Pb bioaccessibility.

Table 7. Total concentration, bioaccessibility and relative bioavailability of Pb in the < 250 μm soil particle size fraction of samples BHK1-12.

Sample	Pb (mg kg^{-1})	Pb Bioaccessibility (%) [*]		Pb Relative Bioavailability (%)	
		SBRC-G	SBRC-I	Mean	Standard Deviation
BHK1	9930 \pm 468	77.2 \pm 2.8	20.1 \pm 1.0	-	
BHK2	3670 \pm 178	69.1 \pm 3.5	25.3 \pm 1.1 ^b	15.4	3.0
BHK3	6416 \pm 453	70.0 \pm 4.7	20.8 \pm 1.6 ^b	6.8	1.2
BHK4	2107 \pm 66	42.7 \pm 7.1	20.5 \pm 1.4 ^b	10.4	1.4
BHK5	3536 \pm 51	66.7 \pm 3.0	22.6 \pm 0.5 ^a	18.8	3.9
BHK6	582 \pm 12	66.3 \pm 2.7	12.0 \pm 5.4 ^a	15.9	2.8
BHK7	316 \pm 1.0	64.0 \pm 1.5	4.8 \pm 0.3 ^c	33.3	8.0
BHK8	267 \pm 14	41.5 \pm 2.6	1.9 \pm 0.2	-	-
BHK9	452 \pm 18	54.6 \pm 2.5	5.4 \pm 0.2 ^c	44.8	5.1
BHK10	1572 \pm 16	79.5 \pm 3.0	14.2 \pm 0.8 ^a	15.9	2.6
BHK11	1097 \pm 15	71.7 \pm 2.6	19.2 \pm 0.3 ^b	11.2	2.8
BHK12	1312 \pm 42	61.7 \pm 1.6	19.5 \pm 0.7 ^a	16.9	3.9

*Values represent the mean and standard deviation of triplicate analysis.

^aNo significant difference ($p > 0.05$) between SBRC-I and Pb relative bioavailability.

^bSBRC-I is significantly higher ($p < 0.05$) than Pb relative bioavailability (i.e. over-prediction of Pb relative bioavailability).

^cSBRC-I is significantly lower ($p < 0.05$) than Pb relative bioavailability (i.e. under-prediction of Pb relative bioavailability).

Conclusions

Assessment of Broken Hill soil using an in vivo mouse model determined that Pb relative bioavailability was significantly lower than the value used to derive the Pb HILa value in the NEPM. For 8 of the 10 soils assessed, Pb relative bioavailability was < 20%, although for BHK7 and 9, Pb relative bioavailability was 33.3 and 44.8% respectively. For the complete dataset (10 soils), the upper 95% confidence intervals of the mean for Pb relative bioavailability was 21.5%. Utilisation of data of this nature will have significant impact on the prediction of blood Pb levels using the Integrated Exposure Uptake Biokinetic Model (IEUBK) through the refinement of the default absolute bioavailability parameter (in the current iteration of the NEPM, the default Pb absolute bioavailability value is 50% = 100% relative bioavailability).

When Pb bioaccessibility was assessed using the SBRC assay, poor relationships were observed when in vivo and in vitro values were compared. The gastric phase of the SBRC assay produced values that were up to 10-fold higher (1.2-10.3) compared to the corresponding Pb relative bioavailability value. When the SBRC intestinal phase was assessed, there was no significant difference ($p > 0.05$) between SBRC intestinal phase values and Pb relative bioavailability for 4 soils (BHK 5, 6, 10, 12), while for BHK2, 3, 4 and 11, in vitro assessment over-predicted Pb relative bioavailability by 1.6-3.1 fold. However, where elevated Pb relative bioavailability was observed (BHK7, 9), assessment of Pb bioaccessibility using the SBRC intestinal phase, significantly underestimated Pb relative bioavailability. While bioaccessibility assay have the potential to be utilised as surrogate measures for Pb relative bioavailability, understanding the relationship between in vivo and in vitro data is critical for confidence in the use of surrogate assays for exposure assessment refinement.

Recommendations

1. Variability in Pb relative bioavailability across Broken Hill

This report identified that Pb relative bioavailability on the southern side of the line of lode was significantly lower than the default value used to derive the NEPM HILa value: Pb relative bioavailability ranged from 6.8-44.8%. However, it is unclear whether Pb relative bioavailability from the southern side of the line of lode is typical of values in other parts of Broken Hill. If smelting was more prominent in other Broken Hill locations, conceivably Pb relative bioavailability may be higher due to Pb speciation differences between smelter emissions. Differences in Pb speciation were observed between soil samples from the northern and southern sides of the line of lode as detailed in Table 3. It would be prudent to assess Pb relative bioavailability in other Broken Hill locations, particularly where historic Pb smelting occurred. An alternative approach would be to utilise blood Pb maps to inform sampling locations (i.e. targeting locations where elevated blood Pb levels occur). This may identify whether Pb relative bioavailability is similar across Broken Hill or whether bioavailability 'hotspots' occur; such information is important for refining exposure parameters for IEUBK modelling.

2. Assessment of in situ approaches for minimising Pb relative bioavailability?

For locations where total Pb concentrations (marginally) exceed the 'acceptable' threshold Pb value, in situ treatment to minimise Pb relative bioavailability may be a cost effective risk management strategy. Phosphate amendments to Pb-impacted soil have the potential to decrease Pb relative bioavailability through the in situ or in vivo formation of highly insoluble Pb phosphate minerals (which are poorly absorbed) (see Juhasz et al., 2014). This could be investigated in Broken Hill soil to determine the

efficacy of phosphate amendments to reduce Pb relative bioavailability and their cost effectiveness as a secondary risk minimisation strategy to soil capping.

3. *Linking indoor-outdoor exposure and IEUBK modelling*

The IEUBK model is a multicomponent tool for predicting childhood blood Pb levels based on a number of exposure assumptions. Where available, site specific data may be utilised to refine default assumptions. However, as with any model, the strength of the prediction is dependent on the validity or robustness of the input data. Although many default assumptions were adjusted during the revision of the NEPM in 2013 (i.e., Pb in water, Pb in food etc.), these parameters have minimal influence on predicted blood Pb levels. Excluding soil-Pb concentration, 5 parameters exert considerable influence on blood Pb modelling:

- I. The predicted indoor dust-Pb concentration which is calculated as a proportion (x 0.7) of the outdoor soil-Pb concentration.
- II. The proportion of outdoor soil and indoor dust contributing to total incidental ingestion (in the NEPM it is 50:50).
- III. The amount of outdoor soil and indoor dust ingested (default assumption of 100 mg day⁻¹).
- IV. The bioavailability of Pb in outdoor soil (absolute bioavailability of 50%).
- V. The bioavailability of Pb in indoor dust (absolute bioavailability of 50%).

Refining points II and III is difficult as these are dependent on behavioural aspects of the individual, while this report contributes soil-Pb relative bioavailability data for locations on the southern side of the line of lode (i.e. parameter IV). For Broken Hill blood Pb modelling, factors contributing to indoor exposure are largely unknown. This offers significant challenges for the IEUBK model as a predictive tool. It would be prudent to undertake an assessment of indoor dust to quantify Pb concentration and to identify Pb sources (considering Pb-paint) and speciation with comparison to outdoor soil-Pb concentrations in various particle size fractions. As Pb source, speciation and particle size are influential factors controlling Pb solubility in vivo, an assessment of Pb relative bioavailability in indoor dust is recommended (as it may differ to soil) in order to strengthen assumptions and robustness of IEUBK blood Pb predictions.

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